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The objective of this project is to develop improved dose forms for the delivery of pain management agents. Given a military scenario where rapid deployment of Special Operations Forces demands effective medical resources, the need for therapeutic pain management tools is obvious. Pain relief is critical in maintaining the ability of the field soldier to defend and protect himself particularly in cases where mobilization is not imminent. Our development work on a pain management system, of which a hydromorphone(HM)/poly(lactide-co-glycolide) (PLGA) delivery system is a part, addresses battlefield treatment with respect to personnel maintenance. A long-acting HM/PLGA implant, easily implanted via a loaded trocar, could provide systemic relief from pain at the time of injury. Phase I work included in vitro and in vivo (mice) testing of four HM/PLGA matrices, HM at 25% and 50% loading in each of two PLGA's (85:15 and 50:50). In vitro release data and mouse tail flick data permitted selection of a system for further development - a 50:50 PLGA containing 25% HM. Data support its use as a 10-day pain management system for combat casualty care.

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Joseph D Gresser 10 Oct 1996

PI - Signature Date

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EXECUTIVE SUMMARY

The objective of this project is to develop improved dose forms for the delivery of pain management agents. Given a military scenario where rapid deployment of Special Operations Forces demands effective medical resources, the need for therapeutic pain management tools is obvious. Pain relief is critical in maintaining the ability of the field soldier to defend and protect himself particularly in cases where mobilization is not imminent. Our development work on a pain management system, of which a hydromorphone(HM)/poly(lactide-co-glycolide) (PLGA) delivery system is a part, addresses battlefield treatment with respect to personnel maintenance. A long-acting HM/PLGA implant, easily implanted via a loaded trocar, could provide systemic relief from pain at the time of injury.

Phase I work included in vitro and in vivo (mice) testing of four poly(d,l-lactide-co-glycolide) (PLGA) matrices containing hydromorphone hydrochloride (HMh). In two, containing HMh at 25 and 50% (w/w), the lactide to glycolide ratio of the polymer was 85:15 (PLGA-85:15). The other matrices, containing HMh at the same loadings, were fabricated using a PLGA with a 50:50 lactide:glycolide ratio (PLGA-50:50). In vitro release data, coupled with results of tail flick tests, permitted selection of a system for Phase II work - a PLGA-50:50 containing 25% HMh. Linear regression of the release data yielded %Release = 12.89+3.56(days); r = 0.9690. Mice implanted with this system (total controlled release dose = 61.6 mg/kg bw) showed 61% of maximum analgesic response (%MAR) at day 2, declining linearly to 16% at day 8. Linear regression for day 0-8 was %MAR = 89.45-9.40t (r = -0.9646). Excluding the 100% MAR shortly after implantation on day 0, the linear regression yielded 74.02-6.96t (r = 0.9905).

These in vitro release data and mouse tail flick data permit selection of a system for further development - a 50:50 PLGA containing 25% HMh. The development is a 10-day pain management system for combat casualty care.

SECTION 1
INTRODUCTION

1.1 Project Objectives

The objective of this SBIR project is to develop improved dose forms for the delivery of pain management agents. The amelioration of post-operative pain or pain associated with injury when treated with potent narcotic analgesics may be associated with sedation and possible side effects such as dizziness and nausea. Thus functional activity may be reduced for as long as pain relief is necessary. Nevertheless the opiate analgesics have an important role which cannot always be filled by local anesthetics. When pain is severe and long lasting it may be desirable to couple a narcotic with a local anesthetic. The side effects may be minimized and long lasting relief may be achieved by controlled release implants for delivery of either or both types of agent. Anesthetic formulations may be implanted at the wound or surgical site for local delivery of drug. Analgesic formulations may likewise be implanted or implanted at alternative, non-traumatized sites for systemic delivery of drug. By use of a biodegradable excipient, poly(lactide-co-glycolide) (PLGA), the implants need not be removed and drug delivery can be adjusted to address targeted deliveries from several days to about two weeks using one long-acting dose form.

Controlled release pharmaceutical dosage forms may offer one or several advantages over conventional dosage forms of the same drug. These include reduced dosing frequency, decreased incidence and/or intensity of adverse effects, greater selectivity of pharmacologic activity and a more constant therapeutic effect. While it is not true that controlled release products have significant advantages in all cases, controlled release systems are therapeutically advantageous primarily for certain subpopulations of patients where, for example, patient compliance or logistics of drug administration are issues. This latter point coincides with the need to address efficient combat casualty care. Given a military scenario where rapid deployment of Special Operations Forces demands effective medical resources, the need for therapeutic pain management tools is obvious. Pain relief is critical in maintaining the ability of the field soldier to defend and protect himself particularly in cases where mobilization is not imminent. Our development work on a pain management system, of which a hydromorphone/PLGA delivery system is a part, addresses battlefield treatment with respect to personnel maintenance.

The agents chosen for our overall development of a pain management system are the narcotic analgesic, hydromorphone, and the local anesthetic, bupivacaine. The implants are short cylindrical rods, the number of which can be chosen to deliver an appropriate dose. The high potencies of both bupivacaine and hydromorphone permit implantation of relatively small volumes of material. The rationale for a dual or combination delivery system lies in the recognition that an opioid when, as an example, used for epidural or intrathecal administration may not provide sufficient analgesia for many patients, but in combination with a local anesthetic such as bupivacaine appears to induce analgesia of more rapid onset and duration and reduce the dose of anesthetic required. With the decrease in dosage, anesthetic associated hypotension and muscle weakness are lessened (Drug Evaluations Annual, 1995).

1.2 Phase I Statement of Work

The overall objective of our work is to develop controlled release implants for relief of pain. In Phase I of this SBIR, the analgesic hydromorphone was proposed for incorporation into a biodegradable excipient designed for continuous release for one to several weeks. The proposed relatively short duration minimizes the possibility of dependence and permits flexibility in dosing by recognizing that delivery for longer periods may not be necessary. The excipient uses a modified poly(lactic-co-glycolide) acid, PLGA, prepared as a low density material with which the drug is combined. The polymer/drug matrix is then extruded under high pressure to produce a high density nonporous dose form. In Phase I, *in vitro* release rates and *in vivo* efficacy in small animals was evaluated against the following variables controlling drug delivery: (1) lactide: glycolide copolymer ratio, and (2) drug loading.

Several factors were involved in the choice of an analgesic for systemic controlled release via an implant. It is obvious that the delivery system must be physically small to minimize trauma. And, the analgesic must be active at low serum concentrations and require low doses to achieve this concentration. The requirement of high potency allows preparation of delivery systems with small quantities of drug. It is also desirable that the elimination half-life be fairly short to avoid chronic toxicity. Chronic toxicity may develop on repeated exposure (via controlled release or multiple doses) over a long period to an agent whose rate of entry is greater than the rate of elimination.

Hydromorphone (HM) is a semisynthetic opioid analgesic which meets these criteria. It is sold as the hydrochloride under the trade name Dilaudid. A dose of 1.5 mg can achieve a 50% elevation of the pain threshold and it has a potency about seven-fold greater than morphine (Levine, 1973). Relevant pharmacokinetic data for hydromorphone is presented below (Clark et al., 1992).

Hydromorphone Pharamacokinetics		
Dose by injection	(D)	1.5 mg
Duration of effect	(td)	4-6 hours
Renal elimination		6%
Volume of distribution	(Vd)	2.9 liters/kg
Elimination half-life	($t_{1/2}$)	2.4 hours
Clearance	(Cl)	15ml/min/kg
Steady State Concentration	(Cpav)	4.4 ng/ml

These data allow calculation of the rate of delivery required to achieve the necessary serum levels for satisfactory pain control. The desired serum level (initial concentration), given by D/(VdW), is $0.007 \mu g/ml$, where W is taken as 75 kg (a man's weight). The steady state concentration (Cpav), defined by Cpav = (maintenance dose)/(dosing interval)(Clearance) for

100% bioavailability (as would be the case with this implantable system), is 4.4 ng/ml. Knowing the steady state concentration and the clearance allows calculation of the rate of elimination from the body and thus a required delivery rate of $5\mu g/\min$. Thus an injection with a one-week lifetime must contain 50 mg of hydromorphone. At a practical and practicable loading of 25% by weight, the drug/polymer matrix would weigh 200mg.

These estimates appear reasonable. A single dose crossover study of 27 normal subjects showed than an 8 mg tablet (oral delivery) achieved a maximum plasma concentration of 5.5 ng/ml, almost identical to the 5.7 ng/ml observed for an equivalent oral dose taken as a 1mg/ml solution (Physicians Desk Reference [PDR], 1994). This is quite close to the steady state concentration of 4.4 ng/ml calculated above.

As is apparent from the above discussion, hydromorphone is a likely candidate for a controlled release system. Several other analgesics are also available with similar potencies for which similar calculations could be made. As with hydromorphone, the drugs listed below may be delivered intramuscularly (IM) or subcutaneously (SC) (PDR, 1994; Beaver, 1980). Doses listed are equianalgesic to 10 mg IM morphine.

Nonproprietary (trade) name	Dose, mg	Duration Compared with Morphine
Morphine sulfate	10	-----
Hydromorphone HCl (Dilaudid)	1.3-2.0	Slightly shorter
Oxymorphone HCl (Numorphan)	1.0-1.1	Slightly shorter
Levorphanol tartrate (Levo-Dromoran)	2.0-2.3	Same
Butorphanol tartrate (Stadol)	1.5-2.5	Same
Methadone HCl (Dolophine)	10	Same

It is to be noted that recent work with opioid peptides (Silbert et al., 1991; Misterek, 1994) indicates that these are also highly potent analgesics. On the other hand, the choice of hydromorphone for this study was based on several considerations. First, the pharmacokinetic parameters listed above suggested that a controlled release system with the desired duration of efficacy is quite feasible. Second, various forms of the drug (parent base, highly soluble hydrochloride salt, or slightly soluble pamoate salt) can be used to adjust release rates and duration of analgesia (Gresser and Sanderson, 1984). Third, forms are stable as indicated by their reported melting points (base: 266-267°C; hydrochloride salt: decomposes at 305-315°C, evacuated tube) (Merck Index, 1989). As our processing conditions for extrusion rarely exceed 37°C, stability is not a problem for hydromorphone or biologicals.

However, a major consideration in developing a delivery system for a highly potent agent of high polarity, such as with hydromorphone hydrochloride, is that release tends to be rapid and to be associated with what is termed an "early burst" of drug — an initial release within the first hours of what may be a significant fraction of the incorporated drug. In addition to the uncertain therapeutic impact of an "early burst" (vis a vis repeated boli of drug) at the beginning of each dose period, there is the practical import of early reservoir depletion and the attendant need for a mass of matrix which is greater than necessary. We have addressed this problem in our previous work on delivery systems by incorporating a low density polymer excipient under high pressure extrusion conditions (Hsu et al., 1995; under patent to Cambridge Scientific, Inc.).

Thus, the technical goal of Phase I was to demonstrate continuous analgesia for one to several weeks by the sustained delivery of hydromorphone from the biodegradable polymer PLGA. The methods and materials of this feasibility study followed from our basic premise that controlled delivery of hydromorphone can be achieved by incorporating the drug into a low density polymer, the resulting matrix then being subjected to high pressure extrusion. We prepared four HM matrices — a 25 and 50% loading in each of two PLGA polymers (an 85:15 and a 50:50). The four matrices were then tested *in vitro* to compare the relative rates of drug release and *in vivo* to determine the duration of analgesia.

The tasks of the Phase I project can be summarized as follows:

- Task 1. Polymer Selection, Purification, and Characterization
- Task 2. Matrix Preparation
- Task 3 *In Vitro* Measurement of Hydromorphone Release
- Task 4 *In Vivo* (Mouse) Tail Flick Test of System Duration
- Task 5 Supply Samples to Army COTR for Testing
- Task 6 Reporting

SECTION 2
METHODS AND MATERIALS

2.1 Materials

Polymers were purchased from Boehringer-Ingelheim Chemicals, Henley Division, Montvale, NJ:

- Poly(d,l-lactide-co-glycolide)-85:15 (PLGA-85:15): Resomer RG 858, lot 25054 (GMP quality)
- Poly(d,l-lactide-co-glycolide)-50:50 (PLGA-50:50): Resomer RG 506, lot 34034 (GMP quality)

The following solvents and chemicals were used as received:

- Acetone: Fisher Scientific, A949
- Glacial acetic acid: Fisher Scientific, A38-212
- Isopropanol: Fisher Scientific, A451
- Hydromorphone hydrochloride: Sigma Chemical Co., H-5136
- Tetrahydrofuran: Fisher Scientific, T427 (Optima)
- Methylene Chloride: Fisher Scientific, (Optima) D151

2.2 Polymer Purification

PLGA-85:15 was purified by precipitation as follows. A solution containing 5.0 grams of the polymer per 100 ml of acetone was slowly added with continuous stirring to a large excess of isopropanol (IPA); approximately 1 ml of solution was added to 7-10 ml of IPA. The fibrous precipitate was drained and dried at room temperature for two days before further processing. PLGA-50:50 was used as received.

2.3 Polymer Molecular Weight Analysis

Molecular weight distributions were determined by gel permeation chromatography (GPC) on Waters Associates instrumentation. The system components include a 712 WISP Autosampler and 600/600E Multi Solvent Delivery System Controller with dual pumping capacity. The system is equipped with two detectors; a 410 Differential Refractometer and a 486 Tunable Absorbance Detector, the former being used to follow elution of the polymer. Separations were accomplished with two 7.8x300 mm columns, a 100 angstrom styragel and 100 angstrom styragel columns in series. Chromatograms were developed with a tetrahydrofuran mobile phase at a flow rate of 0.75 ml/min. Data was analyzed by a Maxima 8125 software package.

2. 4 Preparation Of Polymer Foam Particulate And Matrix Preparation

Open celled foams were prepared from both polymers by lyophilization of solutions of the polymers in glacial acetic acid (glHAc). The concentration of the PLGA-85:15 solution was 4.24 g/dl. After lyophilization of the frozen solution for 7 days, 3.99 grams of foam were recovered. The density of the foam was approximately 62 mg/cm3.

A PLGA-50:50 solution containing 5.00 g/dl was also lyophilized for 7 days from which 4.83 grams of foam (approximate density = 70 mg/cm3) was recovered.

Both foams were cryogenically ground in a Tekmar A-10 Analytical Mill equipped with a cryogenic well enabling particle size reduction at liquid nitrogen temperature. Grinding was done for 8-10 minutes at 20,000 rpm followed by sieving in a mechanical shaker to retain particles less than 125 microns.

Each of the sized polymers was combined with the hydromorphone hydrochloride (HMh) to yield mixtures containing 25% or 50% by weight of the latter, for a total of four matrix mixtures. The mixtures were then blended on a rotary mill (without grinding aids) for 24 hours to assure homogeneity. A control of PLGA-50:50 with no HMh was also included.

The HMh/polymer blend was then loaded into a mold equipped with a 1.0 mm conical die, a 1" diameter ram, and external heating tapes. Extrusions were performed under pressure using a Compac Type MRP 40-1 hydraulic press. This press is capable of maintaining constant pressure on the sample. A constant force of 14 tons was maintained on the ram (35,650 psi). The four matrices were extruded at the following temperatures:

PLGA-85:15/25%HMh	52.5-54.2°C
PLGA-85:15/50%HMh	53.9-56.1°C
PLGA-50:50/25%HMh	53.9-55.2°C
PLGA-50:50/50%HMh	51.8-53.9°C
PLGA-50:50 (Control)	51.1-52.6°C.

The resulting cylindrical extrudated were then cut to appropriate lengths for in vitro and in vivo testing.

2.5 In Vitro Testing

HMh has an accessible UV/Vis absorption band between 260-300 nm in which PLGA's are quite transparent. The absorption maximum is at 280.1 nm and has an extinction coefficient of 4.059 AU/mg/ml (AU = absorbance units). Analyses were run in triplicate in 10.0 ml of phosphate buffered saline (PBS) adjusted to pH 7.4. The buffer was contained in 50 ml test tubes which were placed in a thermostatted shaker bath (Precision Model 25) set at 37°C. Aliquot volumes were removed periodically for spectral analysis (Cary 1 UV/Vis spectrometer, Varian Assoc) and then returned to the 50 ml test tube. The percent release was calculated from the relationship

$$\% \text{ release} = (A/e)(V)/wf$$

where A = absorbance at 280.1 nm
e = extinction coefficient = 4.059 AU/mg/ml
V = volume of buffer = 50 ml
w = weight of sample, mg
f = weight fraction of HMh in the sample.

2.6 In Vivo Testing

Male mice (C57 Black/6J) (Jackson Laboratories) weighing 20-25 grams each (6-8 weeks old) were acclimatized for one week prior to initiating the tests. Ten mice, housed five to a cage, were used to test one dose of each formulation and the control (PLGA-50:50). Under methoxy fluorane anesthesia, mice were implanted in the scapular region with matrices cut to deliver approximately 70 mg/kg to a 20 gram mouse. Average doses based on 20 grams are given in Table 1.

Table 1:PLGA-HM Matrices For In Vivo Testing

Description	*Sample ID*	*Length, mm*	*Diameter, mm*	*Weight, mg*
PLGA-85:15/50%HM	76-7-1/10	10.12±0.33	0.71±0.03	2.54±0.13
	76-7-11/20	10.24±0.22	0.71±0.03	2.54±0.32
PLGA-85:15/25%HM	76-10-1/10	5.04±0.73	1.11±0.02	6.10±0.21
PLGA-50:50/50%HM	76-9-1/10	5.15±0.29	0.87±0.03	3.31±0.26
	76-9-11/20	5.14±0.36	0.88±0.02	3.52±0.24
PLGA-50:50/25%HM	76-12-1/10	5.10±0.26	0.96±0.03	4.93±0.32
PLGA-50:50/0%HM	76-13-1/10	4.91±0.26	1.13±0.17	6.53±2.33

Extrusion conditions:

Sample ID	*Force, tons/1"dia. ram*	*Temp., °C*	*Density, g/cm³*
76-7-1/20	14	53.9-56.1	0.63
76-10-1/10	14	52.5-54.2	1.36
76-9-1/20	14	51.8-53.9	1.21
76-12-1/10	14	53.9-55.2	1.36
76-13-1/10	14	51.1-52.6	1.42

Average dose delivered to 20 gram mice:

76-7-1/20	63.5 mg/kg
76-10-1/10	76.3 mg/kg
76-9-1/20	80.5 mg/kg
76-12-1/10	61.6 mg/kg
76-13-1/10	0.0 mg/kg (control)

The justification for the chosen dose is derived from comparison of the dose of morphine used in an earlier study conducted by us of controlled release of naltrexone in which morphine was used as a challenge. In that study, a dose of 7.5 mg/kg was used to challenge naltrexone blockage of morphine analgesia. Assuming the same ratio of potencies of hydromorphone and morphine apply to mice as to humans, approximately 1 mg/kg of hydromorphone delivered ip would be required to induce analgesia. The duration of analgesia can be assumed to be 5 hours. Thus for a two week system with constant delivery, the total dose would be 67.2 mg/kg. The doses used in the present study approximate this.

The standard tail flick test was used to determine the extent and duration of analgesia. Measurements were taken prior to implantation to establish base line data and following implantation at 0.5 hrs, and subsequently at days 2, 4, 7, and 8 or 9. The percent of maximum analgesic response (% MAR) was calculated from the following equation (Silbert et al., 1991).

$$\% \text{ MAR} = [(\text{test latency - control latency})/(\text{cutoff - control latency}]100$$

Test latency is defined as the time required for implanted animals to respond to the heat stimulus. Control latency refers to the pre-implant latency of the test animals. The cutoff is the maximum possible duration of the stimulus as set by the investigator to prevent injury to the animal. (The cutoff time was set at 6.2 seconds.) This test is well described in the literature (Dewey and Harris, 1975).

A preliminary test to check for the possibility of development of tolerance was run prior to initiating the larger series of tail flick tests. A group of 5 mice each was implanted with matrix 76-7 (PLGA-85:15/50%HMh). Another group without implants served as controls. Tail flicks were run on each group over a period of 8 days. In order to differentiate between exhaustion of the implant and development of tolerance, we then conducted a dose response test with the same implanted and control mice. The mice were injected with 0.5 mg/kg of HMh at time t = 0 and tested by tail flick 20 minutes later. After the test (at t = 25 min.) they were given a second 0.5 mg/kg injection and again tested after 20 min. A third tail flick was conducted but without a third dose of HMh.

SECTION 3
RESULTS AND DISCUSSION

3.1 Molecular Weight Analyses of PLGA-85:15 and PLGA-50:50

Four hydromorphone controlled release systems were evaluated in mice in Phase I. The hydrochloride salt (HMh) was incorporated into two poly(d,l-lactide-co-glycolide)s with lactide:glycolide ratios of 85:15 and 50:50 (PLGA-85:15 and PLGA-50:50). HMh was incorporated into each polymer at loadings of 25 and 50% by weight.

Molecular weight measurements by gel permeation chromatography (GPC) were made on both polymers before implantation, after foam formation, and on selected *in vivo* samples after excision form test animals. GPC's were performed on a μ-Styragel column using a tetrahydrofuran mobile phase. Our results were compared with molecular weight data supplied by Boehringer-Ingelheim (BI). Results are presented in Table 2.

Table 2: Weight Average Molecular Weights of Polymers
Before Implantation and After Excision

Polymer	BI	As Rec'd.	Foam	After Excision
PLGA-50:50	98,000	96,410	75,410	Sample 76-13 (controls) 45,060 at nine days
PLGA-85:15	232,000	177,840	153,380	Sample 76-10 (25% HM) 118,360 at nine days
				Sample 76-7 (50% HM) 126,870 at eight days

Our GPC result for the as received PLGA-50:50 weight average molecular weight (Mw) agrees closely with BI's reported value (96,400 vs 98,000). After formation of the foam the Mw had dropped to 75,400, a decrease of 21.8%. The foam was prepared by lyophilization of a glacial acetic acid solution of the polymer. Because foam formation is carried out under anhydrous conditions, the decrease in molecular weight is not due to hydrolysis, but rather, we believe, to transerterification during solution of the polymer according to the following reaction:

$$R-CO-O-R' + HOCO-CH_3 \rightarrow R-CO-OH + CH_3-CO-O-R'$$

where R represents continuation of the polymer chain and $HOCO\text{-}CH_3$ is acetic acid.

Sample 76-13 implants (PLGA-50:50 controls) were excised 9 days after implantation at which time the Mw had dropped to 45,060, a further decrease of 40.2%. This is attributed to PLGA *in vivo* hydrolysis.

Our Mw for the as received PLGA-85:15 (177,840) was 40.2% lower than that reported by BI (232,000). A decrease of 13.7% from our value to 153,400 was recorded after foam formation. After excision the Mw of sample 76-10 (initially containing 25% HM, and excised after 9 days) had fallen by 22.8% to 118,400, while Sample 76-7 (initially containing 50% HM, and excised after 8 days) had fallen by 17.3% to 126,900. The *in vivo* decrease of Mw for both polymers is consistent with known degradation rates: the less crystalline PLGA-50:50 is known to undergo hydrolytic degradation more rapidly than the PLGA-85:15.

3.2 In Vitro Release of Hydromorphone

In vitro release was measured in phosphate buffered saline at 37°C using the UV absorbance at 280 nm. Release curves are shown in Figure 1. Both PLGA-85:15/50HMh and PLGA-50:50/50HMh released virtually all of the drug within 2 days while polymers loaded with 25% drug released more evenly. PLGA-85:15 showed very little release until day 3. From day 3 to day 15, 32% of the drug had been released. PLGA-50:50/25HMh did not show a delayed release. By day 15, 65% of the drug was released. Thus, in these systems, *in vitro* and *in vivo* release correspond closely. The *in vitro* and *in vivo* behavior of the PLGA-50:50/25%HMh has led to choice of this system for further development. Cumulative in vitro release for the four systems is tabulated in Table 3.

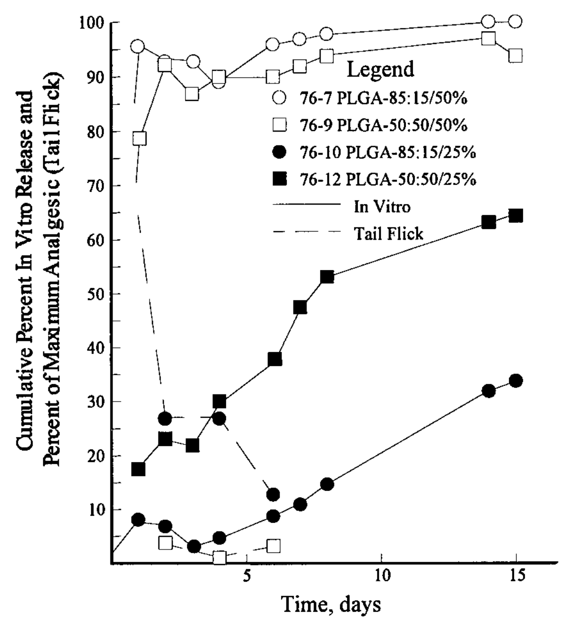

Figure 1. ***In Vitro*** **Release of Hydromorphone from PLGA/HM Implants**

TABLE 3: In Vitro Release: Cumulative Percent Vs Time

Sample	76-7	76-10	76-9	76-12
Polymer	PLGA-85:15	PLGA-85:15	PLGA-50:50	PLGA-50:50
Wt.% HM	50%	25%	50%	25%
Sample Wt., mg	4.03±0.42	6.60±0.70	3.97±0.21	4.93±0.45
Time, days	*Cumulative Percent Release*			
1	96.0±6.1	8.3±1.5	79.3±12.4	17.3±3.2
2	92.7±7.2	8.0±1.0	92.3±1.2	23.7±1.2
3	92.7±5.5	3.3±1.2	87.7±3.1	23.0±1.0
4	89.3±9.5	4.7±0.6	89.7±3.1	28.7±1.5
7	89.3±9.5	8.7±0.6	90.0±1.0	38.7±2.1
8	96.7±6.4	11.7±0.6	92.3±2.5	48.0±2.6
9	97.7±6.4	15.0±1.0	95.0±2.6	53.7±4.9
14	99.0±7.8	31.3±3.5	97.0±3.6	64.3±5.5
15	99.3±7.6	32.0±3.6	94.7±4.0	65.0±5.5
16	92.3±6.7	39.7±11.5	89.7±1.5	63.0±4.6
18	99.7±2.9	47.7±5.1	96.7±1.2	74.3±1.2

3.3 In Vivo Tail Flick (Mice)

Groups of ten mice (male, B5AF1/J, 6-8 weeks old) were implanted subcutaneously with extruded rods containing HMh. Rods, doses delivered, and percent analgesia are described in Table 2. A practical consideration governed the magnitude of the dose implanted via these controlled release systems. The total implant weights varied between 2.5 and 5.0 mg. This was the smallest size which could maintain the cylindrical geometry of the extruded dose form. Thus the weight of HM contained in the rods was 1.2-2.0 mg.

Design of an HM controlled release system for humans is based on the accepted dose of 1-2 mg (say 1.5 mg) delivered by injection every 4-6 hours (say 5 hours). For continuous delivery over a 10 day period, the weight of HM would be (1.5 mg/dose)(24/5 doses/day)(10 days) = 72 mg. Based on the results presented below the recommended system will contain 25% by weight of HM giving an implant weight of $72 \times 4 = 288\text{mg}$.

The analgesic response as measured by the tail flick test (Figure 2) to PLGA-50:50/25%HM and PLGA-85:15/50%HM are virtually identical and show measurable although decreasing analgesia to day 8 at which time analgesia was 16-18% of maximum. We believe that a short term implant should show decreasing analgesia with time rather than a continuing high level of analgesia followed by an abrupt drop. Decreasing analgesia allows the physician to judge the severity of pain with time. Tail flick results are presented in Table 4 as latency (seconds) and as percent of maximum analgesic response.

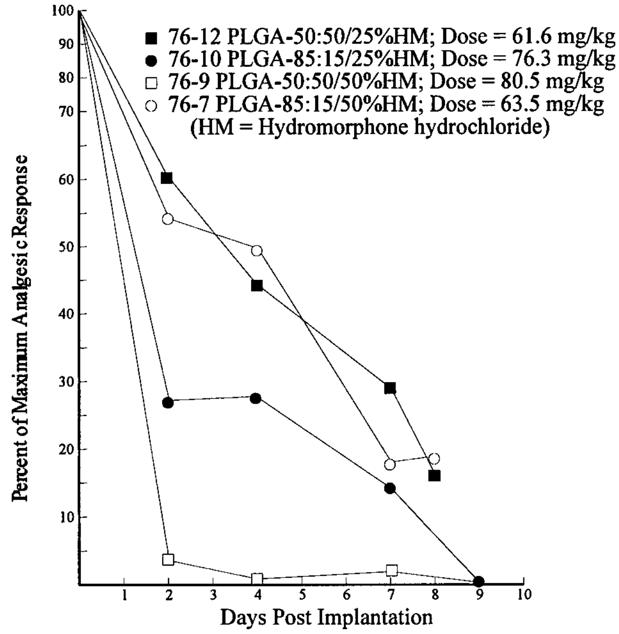

Figure 2. Analgesic Response of PLGA/HM Implants Measured by Tail Flick Response

Table 4: Degree of Analgesic Response by Controlled Release of Hydromorphone Hydrochloride by Tail Flick Test

Sample ID	Day(Date)	Mean D, Sec.	% Maximum Analgesia
76-12-1/10	0(7/15)	3.94 ± 0.17	100
Cages G, H	2(7/17)	2.39 ± 0.31	60.7 ± 7.9
Dose: 61.6 mg/kg	4(7/19)	1.75 ± 0.35	44.4 ± 8.9
PLGA-50:50/25%HM	7(7/22)	1.12 ± 0.22	28.2 ± 5.6
	8(7/23)	0.63 ± 0.12	16.0 ± 3.0
76-9-1/10	0(7/1)	3.44(1)	100
Cages E,F	2(7/3)	0.15 ± 0.10	4.4 ± 2.9(2)
Dose: 80.5 mg/kg	4(7/5)	0.04 ± 0.04	1.2 ± 1.2
PLGA-50:50/50%HM	7(7/8)	0.09 ± 0.05	2.6 ± 1.5
	9(7/10)	0.0	0.0
76-10-1/10	0(7/1)	3.44(1)	100
Cages C,D	2(7/3)	0.93 ± 0.37	27.0 ± 10.8 (3)
Dose: 76.3 mg/kg	4 (7/5)	0.94 ± 0.40	27.3 ± 11.6
PLGA-85:15/25% HM	7(7/8)	0.46 ± 0.23	13.4 ± 6.7
	9 (7/10)	0.0	0.0
76-70-1/5	0(6/17)	3.73 ± 0.24	100
Cage A	2 (6/19)	2.01 ± 0.59	53.9 ± 15.8
Dose: 63.5 mg/kg	4 (6/21)	1.83 ± 0.22	49.1 ± 5.9
PLGA-85:15/50% HM	7(6/24)	0.66 ± 0.28	17.7 ± 7.5
	8(6/25)	0.69 ± 0.39	18.5 ± 10.5

(1) One mouse in Cages C,D did not have an implant. Thus the mean time 0 D-value for cages C,D (3.24 ± 0.38) and cages E,F (3.65 ± 0.38) was used.

(2) This formulation released HM very rapidly causing animals to sweat profusely and lowering body temperature to a mean of $34.3 \pm 0.37^{o}C$ at 5 hrs. post implantation.

(3) Body temperature as normal at 5 hrs post implantation ($38.2 \pm 0.33^{o}C$).

Both polymers have advantages and disadvantages as delivery vehicles. The PLGA-50:50 is more rapidly resorbed: although a desirable feature, the possibility of dumping (a period of rapid release) at some point is increased.

Although less rapidly absorbed, release from the PLGA-85:15 is more easily controlled, except at high loadings. Thus the slow *in vitro* release of HM from the 25% loaded PLGA-85:15 resulted in insufficient analgesia. Insufficient analgesia from the 50% loaded PLGA-50:50 was, on the other hand, due to the early dumping and exhaustion of the HM. Thus these two systems can be eliminated from further consideration. The PLGA-85:15 containing 50% HM, although giving good analgesic response, also released too rapidly. Thus we can also eliminate this system.

The remaining system, PLGA-50:50 containing 25% HM gave both good analgesic response coupled with acceptable *in vitro* release kinetics. It also has the virtue of being more rapidly resorbed. It is this system which will be the major focus of Phase II.

3.4 In Vivo Testing for Tolerance

Prior to initiating the tail flick tests, a test for development of tolerance was conducted with two sets of five mice each (Shuster et al., 1963). One set was implanted with Matrices 76-7 (PLGA-85:15/50% HM), the other set served as controls. Tail flicks were run on each set over 8 days. The results as percent of maximum analgesia are given below.

Time	3 hours	day 2	day 4	day 7	day 8
Test	100	59	50	19	18
Controls	no change in response time				

These results show a large reduction in response to HM over the 8 days. It also shows that the implants were releasing during this time. In order to differentiate between exhaustion of the implant and development of tolerance, we then conducted a dose-response test with the same implanted and control mice. The mice were injected with 0.5 mg/kg HM at time $t = 0$ and tested by tail flick 20 minutes later. After the test (at $t = 25$ min.) they were given a second 0.5 mg/kg injection of HM and again tested after 20 minutes. A third tail flick was performed but without a third dose of HM. Results are reported below as percent of maximum analgesic response. It is important to bear in mind that the implanted mice had an 18% residual analgesic response at the beginning of this test so that this must be added to the analgesic response developed in the dose response test.

Test	Total HM Dose, mg/kg	Implanted Mice % max.	(+18% Resid.)	= Total	Controls
1	0.5	42	(+18)	= 60	64
2	1.0	79	(+ 18)	= 97	89
3	1.0	72	(+ 18)	= 90	91

Thus the implanted mice and the control mice show about the same response to HM challenge, and by this test we can conclude that no tolerance developed over eight days of exposure to the implants. The implant appears to have continuously released HM during this period as evidence by the continued analgesic response over the eight days.

3.5 Extraction of HM from Excised Rods

A simple extraction procedure was tested and used to analyze residual HM in rods excised from the test animals. Rods were cleaned, dried, combined in groups of 5 and weighed. These were then dissolved in methylene chloride and then extracted with 25 ml of 1M HCl. Ultraviolet absorbance of the aqueous layer was used to calculate the remaining hydromorphone. The two groups of PLGA-50:50 containing 50% HMh were completely exhausted but the two groups of

PLGA-50:50 containing 25% HMh contained 36.5 ± 3.5% of the original content. Fresh rods were used to confirm this analysis: results agreed within ±4% of the expected value.

SECTION 4
FUTURE RESEARCH AND DEVELOPMENT: PLANS FOR PHASE II

The amelioration of post operative pain or pain associated with injury when treated with potent narcotic analgesics may be associated with sedation and possible side effects such as dizziness and nausea. Thus functional activity may be reduced for as long as pain relief is neccessary. Nevertheless the opiate analgesics have an important role which cannot always be filled by local anesthetics. When pain is severe and long lasting it may be desireable to couple a narcotic with a local anesthetic. The side effects may be minimized and long lasting relief may be achieved by controlled release implants for delivery of either or both types of agent. These formulations may be implanted at the wound or surgical site. By use of a biodegradable excipient, poly(lactide-co-glycolide) (PLGA), the implant need not be removed. Drug delivery can be adjusted from several days to about two weeks. Although a dual drug controlled release system may eventually be considered, the agent chosen for Phase II development is limited to the narcotic analgesic, hydromorphone. The implants are short cylindrical rods, the number of which can be chosen to deliver an appropriate dose. The high potency hydromorphone permit implantation of a relatively small volume of material.

Rats and sheep are the species selected for confirmation of the controlled release formulation selected in Phase I. Concurrent with animal testing GMP protocols will be developed for preparation of samples to be tested in limited Phase I clinical trials in the second year of the program.

Sheep are the choice for the larger species testing. Nolan et al. (1987) clearly state the advantages of sheep: "Sheep have the advantage of adapting well to handling and housing and can be easily trained to stand in a small pen, with apparatus attached to them. They are fairly docile and not inclined to inflict injury on their handlers, and they are large enough to allow serial sampling of body fluids for pharmacokinetic studies." Livingston et al. (1985, 1992) have used a heat source as the pain stimulus for studying the effects of antinociceptive drugs. The heat is applied over a small area via a small clip-on device attached to the pinna of the sheep's ear. The temperature is increased at a controlled rate until the pain threshold is reached at which point a flick of its head activates an inertia cut-off switch which deactivates the heat source and timer. Normal head motions are not rapid enough to operate the cut-off switch. Both the final temperature and the time to cut-off are registered. Figures 3 and 4 show the device in place on the pinna and the circuitry (both figures taken from Nolan et al., 1987).

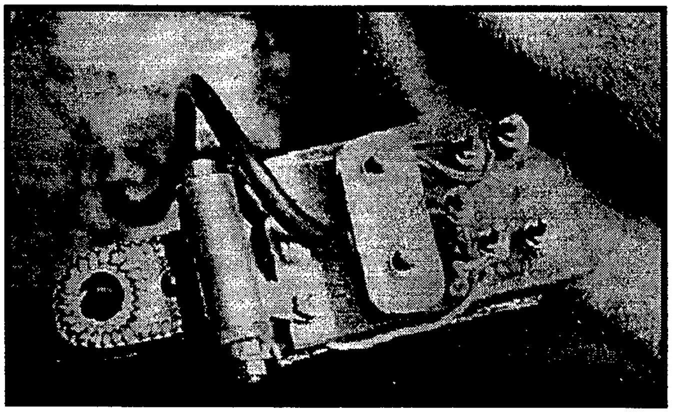

Figure 3. Thermal Threshold Clip Placed on the Pinna of the Ear of a Sheep (Nolan et al., 1987)

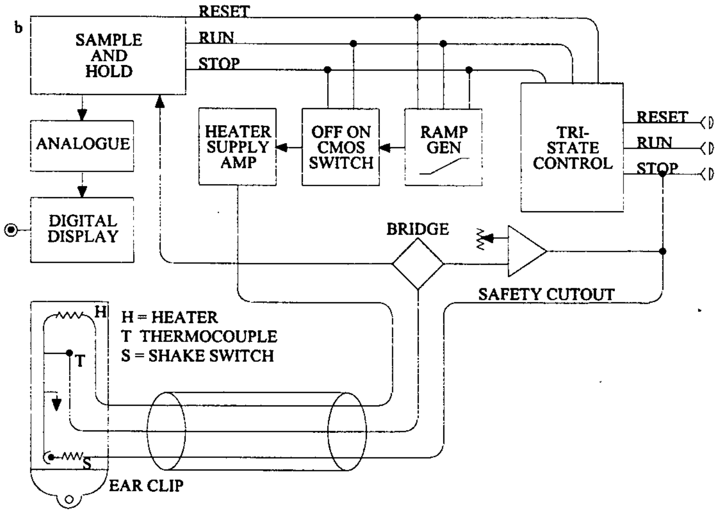

Figure 4. Schematic Diagram of Thermal Threshold Clip Circuit (Nolan et al., 1987)

Phase II is primarily directed toward Phase I clinical trials which will follow the preclinical animal studies mentioned above. To this end Good Manufacturing Practices (GMP) protocols will be developed for preparation of implants. An application for an Investigational New Drug (IND) will be drafted. The Phase 1 clinical investigation aims to evaluate the pharmacokinetic characteristics and initial safety of the controlled release system in ten healthy adult volunteers. The clinical trial will be conducted at the New England Medical Center (NEMC) Hospital, Boston, MA. The Responsible Physician is Dr. Daniel B. Carr of NEMC.

SECTION 5
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SECTION 6
PROJECT PERSONNEL

The following personnel contributed to the successful execution of this Phase I SBIR project:

Cambridge Scientific, Inc.

- Joseph D. Gresser, Ph.D. (Principal Investigator)
- William A. Apruzzese
- Debra J. Trantolo, Ph.D.
- Donald L. Wise, Ph.D.

Collaborators

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